Effects of Ischemia Duration on Neurological Outcome, CA1 Histopathology, and Nonmatching to Sample Learning in Monkeys

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Background and Purpose: Male cynomolgus monkeys (n=10) were subjected to varying durations of global cerebral ischemia to determine the relation between dose (ischemic duration) and response (outcome).

Methods: Each monkey was anesthetized with halothane, and global cerebral ischemia was produced by a neck tourniquet and trimethaphan-induced hypotension. The animal was subjected to 3 (n=3), 9 (n=3), or 12 (n=4) minutes of ischemia. Neurological examinations were performed daily for 30 days or until the monkey was neurologically normal. Approximately 1 month after ischemia, the animal was evaluated for evidence of neurobehavioral abnormalities with the nonmatching to sample test. When testing was complete, the monkey was killed with an overdose of pentobarbital and the brain perfused with formalin and removed for histopathologic analysis, with particular attention devoted to the hippocampal CA1 region.

Results: Monkeys subjected to 3 or 9 minutes of ischemia were neurologically normal (except for a very mild injury in one 9-minute animal) immediately after ischemia and had normal CA1 histology. Monkeys subjected to 12 minutes of ischemia were grossly abnormal neurologically after ischemia, but two of the four animals made a complete recovery (neurological deficit score of 0) by 30 days. Monkeys subjected to 12 minutes of ischemia had mild damage in the CA1 region, with all other brain regions appearing normal. None of the animals had demonstrable decrements in neurobehavioral function as measured by the nonmatching to sample test.

Conclusions: We conclude that neurobehavioral testing after global cerebral ischemia in primates is feasible, but the ischemic time necessary to produce CA1 damage that could potentially be quantified antemortem with the nonmatching to sample test is greater than 12 minutes in cynomolgus monkeys and may produce temporary severe gross neurological abnormalities as well. (Stroke 1992;23:1471-1478)

KEY WORDS • cerebral ischemia • hippocampus • stroke outcome • monkeys

Despite modern mechanical and pharmacological cardiopulmonary resuscitation techniques, neurological outcome following cardiac arrest is still poor even though there appears to be an increased number of overall survivors.1 Neurological deficits after resuscitation may be subtle and involve primarily the ability to acquire new information or learn new tasks. It is thought that this type of deficit may in part be related to selective destruction of the CA1 sector of the hippocampal formation, an area of the brain known to be unusually vulnerable to the effects of even short periods of global cerebral ischemia.2,3

Behavioral psychologists have studied the effects of discrete brain lesions on learning and behavior in primates principally by performing ablation studies of various components of the medial temporal lobe including the hippocampus.3-6 Some of the tests used to evaluate the neurobehavioral effects of ablation lesions in primates are identical to the tests used to evaluate patients with subtle types of amnesia thought to be due to selective injury of the hippocampus that may occur after cardiac arrest.7 This suggests that primates subjected to global brain ischemia with resulting isolated CA1 lesions could be evaluated with neurobehavioral tests and that this might represent a clinically relevant animal model of human cognitive disability following cardiac arrest (or other global ischemic/hypoxic insults). However, it is critically important to determine a duration of ischemia that will produce the necessary pathological changes but still allow the animal to perform the motor tasks associated with memory testing. If duration of ischemia is too great, animals may be left with impaired motor function and be unable to complete tasks that test cognitive abilities.

In an earlier work, it was demonstrated that 15 minutes of global cerebral ischemia in cynomolgus
monkeys led to moderate to severe CA1 damage in all animals and significant quantifiable neurobehavioral deficits compared with historical control animals. However, one of those five monkeys died, presumably from severe stroke, and some of the other animals required a number of days of intensive care after ischemia before eventual neurological recovery. Therefore, we sought to determine the dose–response relation between duration of global cerebral ischemia (dose) and the functional and/or anatomic impairment (response) of the specific brain area to be evaluated (i.e., the hippocampal CA1 area). Although data correlating CA1 damage with duration of ischemia exist for rats and gerbils, there has been no systematic evaluation in primates. It was for this reason that we chose to evaluate primates following increasing durations of global cerebral ischemia with neurological examinations, neurobehavioral testing, and analysis of CA1 histopathology.

Materials and Methods

This protocol was approved by the Animal Subjects Committee of the University of California, San Diego. This model has been described in detail elsewhere using different species of monkeys. Ten male cynomolgus monkeys (Macaca fascicularis) weighing 2.6–5.0 kg were studied. The animals were studied in random order after arriving at our institution and undergoing quarantine procedures. The monkeys had free access to food but received only water for the 24 hours prior to the study. Each monkey was initially anesthetized with 4.0% halothane and O2 in a Plexiglas box. An intravenous catheter was placed in a hind limb vein, and 1.0 mg pancuronium was given intravenously to facilitate tracheal intubation with a 4-mm-i.d., cuffed, wire-reinforced tube. Ventilation was controlled to maintain end-tidal CO2 at approximately 35 mm Hg by adjusting the ventilator rate to about 15 breaths per minute and tidal volume to 12 ml/kg. Anesthesia was maintained with 0.5% halothane and 66% N2O. The monkey's eyes were lubricated and taped closed. Immediately after placement of the intravenous cannula, a blood sample was obtained for analysis of blood glucose concentration (glucose analyzer 23A, Yellow Springs Instrument Co., Yellow Springs, Ohio) and hematocrit. Esophageal and temporalis muscle temperature probes were placed, and temperature was maintained near 37.0°C. An electrocardiogram, the electroencephalogram (EEG) monitored with four electrodes (two frontoparietal, two parieto-occipital), end-tidal CO2 and halothane concentrations, and airway pressure. Additional doses of 0.02 mg/kg pancuronium were administered intravenously as needed to maintain paralysis. Thirty minutes after the start of halothane administration, a 5% dextrose solution in 0.45% saline was administered as an infusion of 13 ml/kg over 15 minutes.

After a sterile preparation, the femoral artery and vein were cannulated with PE-90 catheters. Arterial pressure was monitored continuously, with the base of the skull as the zero reference point. Ventilation and the inspired O2 concentration were adjusted to maintain Pao2 at 150–200 mm Hg and Paco2 at 30–35 mm Hg. The monkeys were positioned supine on the table, and ischemia was begun exactly 75 minutes after infusion of the 13 ml/kg glucose-containing solution was completed.

Before tourniquet inflation the hematocrit, blood glucose concentration, and arterial blood gases were rechecked. Adjustments were made so Pao2 was 150–200 mm Hg and Paco2 was 30–35 mm Hg. Other criteria included blood glucose concentration of 100–200 mg%, base excess of −4 to 4 meq/l, and temporalis muscle temperature of 37±0.2°C. When the above criteria were met, a tourniquet was placed around the monkey's neck in a manner that allowed one finger to fit under the cuff. Halothane was discontinued, and a stopwatch was started. After halothane had been discontinued for 3 minutes, 20–40 mg trimethaphan was given intravenously via the femoral central venous catheter, with the amount given dependent on the initial blood pressure and the monkey's weight. When mean arterial pressure (MAP) decreased below 50 mm Hg, the neck tourniquet was inflated to 1,500 mm Hg and ventilation with xenon-133 in 100% O2 was begun. The tourniquet remained inflated for 3, 9, or 12 minutes. The time required for the onset of an isoelectric EEG was recorded. During the ischemic period, repeated measurements of radiation from the monkey's head were made with a Geiger counter and compared with readings taken from the chest and abdomen to verify cerebral ischemia.

Increases in MAP above 50 mm Hg were controlled with a positive end-expiratory pressure of 0–10 cm H2O and a trimethaphan infusion. Two minutes before the end of ischemia, a norepinephrine infusion was begun as needed to achieve a MAP of 60–80 mm Hg. At the appropriate time, the tourniquet was deflated and removed. The monkey was ventilated with 100% O2, and the norepinephrine infusion was adjusted to achieve a MAP of 80–120 mm Hg.

Following ischemia, a bladder catheter was placed and urine output was monitored. Intramuscular antibiotics (15 mg/kg cefazolin) were administered. Arterial blood gases were checked frequently to assure that normocapnia and Paco2 of >150 mm Hg were maintained, at all times. When continuous EEG activity returned, the monkey was ventilated with 50% N2O in O2. After continuous EEG activity had been present for 1 hour, neuromuscular paralysis was reversed with 0.07 mg/kg neostigmine and 15 μg/kg atropine, and the animal was allowed to breathe spontaneously from a T-piece. If ventilation was adequate as determined by arterial blood gases and the monkey no longer needed the norepinephrine infusion to maintain MAP at >80 mm Hg, monitoring lines were removed using a sterile technique and the animal was transferred to a warmed cage with an oxygen-enriched atmosphere. The trachea of the monkey was extubated when the animal visibly objectied to the presence of the endotracheal tube. Following extubation of the trachea, all monkeys were nursed continuously in a warmed and padded cage until they were able to care for themselves. An animal was turned and suctioned every 4 hours if it was not alert and mobile. Intravenous fluid was given at a rate of 4 ml · kg−1 · hr−1 until the monkey was taking water orally. For some animals, this level of care was required for 1–3 days.

The monkeys were examined daily by two independent observers. Neurological evaluation was carried out
At the time of sacrifice the monkey was deeply anesthetized with ketamine and pentobarbital. The ascending aorta was cannulated, the descending aorta clamped, and the head packed in ice. The animal was then given an overdose of pentobarbital and perfused through the aortic cannula with sequential solutions of 1) cold 1% paraformaldehyde for 2 minutes at a rate of 250 ml/min, 2) cold 4% paraformaldehyde for 10 minutes at a rate of 250 ml/min, 3) cold 4% paraformaldehyde for 50 minutes at a rate of 100 ml/min, and 4) cold 5% sucrose for 20 minutes at a rate of 100 ml/min. The brain was then removed from the skull and blocked for sectioning. Blocks were agitated in cold 10% glycerc, 2% dimethyl sulfoxide (DMSO) overnight, then in cold 20% glycerc, 2% DMSO for a minimum of 3 days before sectioning.

Tissue blocks were rapidly frozen in isopentane chilled in absolute alcohol with dry ice and mounted on the microtome stage. Blocks were kept frozen during cutting by packing with dry ice. Serial coronal sections were cut through the entire hippocampal block at a thickness of 30 μm. Some sections were stored at room temperature in 10% formalin, and some were stored at −20°C in 25% glycerc, 30% ethylene glycol in phosphate buffer. A series of sections approximately 1,000 μm (1 mm) apart were cleared in chloroform and stained with thionin for Nissl substance (approximately 24 sections for each monkey). Two sections from each animal (approximately one third and two thirds of the way through the block) were stained for glial fibrillary acidic protein (GFAP). Sections for GFAP immunohistochemistry were pretreated with chloroform, 0.3% H2O2 in methanol, and Triton X-100. Sections were blocked with 10% normal goat serum, reacted overnight at 4°C with the primary rabbit anti-GFAP antibody (Dako Corp., Carpinteria, Calif.) at a dilution of 1:300 followed by biotinylated secondary goat anti-rabbit antibody (Vector Laboratories, Inc., Burlingame, Calif.) at a dilution of 1:50, reacted with avidin-biotin complex/horseradish peroxidase (Vector), and visualized with aminoethylcarbazole. Sections were lightly counterstained with Mayer's hematoxylin. Negative controls, obtained by replacing the primary antiserum with normal rabbit serum, were stained simultaneously. Normal subpial and subependymal gliosis served as an internal positive control.

All sections were examined by a neuropathologist without knowledge of the treatment groups. All cortical and subcortical areas present in the sections were examined for neuronal loss, gliosis, and any other pathological changes. Monkeys subjected to 12 minutes of ischemia were given an overall hippocampal histopathology score by the neuropathologist on the basis of neuronal loss and degree of gliosis and were ranked from the least to the most hippocampal injury.

The cervical spinal cords of two monkeys (one exposed to 12 minutes of ischemia and the other to 3 minutes) were also removed and placed in 10% buffered formalin for fixation. Multiple levels were embedded in paraffin and cut at a thickness of 6 μm. Sections were stained with hematoxylin and eosin and examined by the neuropathologist for evidence of cell loss, demyelination, or gliosis.

Average neurological deficit scores were compiled for each group of monkeys subjected to the various periods with a 500-point grading scale (Figure 1) used by previous investigators. A neurological deficit score of 0 signified a normal animal and a score of 500 an animal with brain death. Examinations were performed until both observers agreed that the monkey was either neurologically normal or the score on the neurological examination had become stable.

Approximately 30 days following ischemia, after the animals had made maximum neurological recovery, they were introduced to the nonmatching to sample test apparatus. The nonmatching to sample test has been evaluated and described elsewhere. Briefly, this apparatus consists of a cage with a barred window through which the monkey can displace objects covering wells to obtain food rewards. The paradigm is arranged so that the animal is first exposed to an object (known object) and then after a variable waiting period (delay) the animal is exposed to two objects, the known object and a new or novel object. The animal must displace the novel object to retrieve the food reward. Normal monkeys can learn this task and can be trained to achieve a >80% correct response rate even with delays of up to 10 minutes. Monkeys that have bilateral lesions of the hippocampus score at chance, or 50%. It is thought that this test is sensitive to CA1 function because of poor performance by patients who have isolated CA1 lesions. Because of the complete lack of CA1 pathology in monkeys exposed to 3 minutes of ischemia, these animals served as the control group for comparisons on the nonmatching to sample test.
of ischemia for 33 days after ischemia. By this time, the animals were either completely normal or their neurological deficit scores were stable. Only monkeys subjected to 12 minutes of ischemia were neurologically impaired after ischemia, with the exception of one monkey exposed to 9 minutes of ischemia.

After graphing the nonmatching to sample test data, it was obvious that differences between groups might be expected only at the 10-minute delay. Therefore, the average percentage correct for monkeys subjected to 12 or 9 minutes of ischemia at the 10-minute delay was compared with the average percentage correct for the 3-minute (control) group with a one-tailed Student's t test.

Results

Global cerebral ischemia was complete in all 10 monkeys as evidenced by the failure of xenon-133 to gain access to the head during the period of tourniquet inflation. All animals survived the ischemic period; there were no deaths. All criteria with regard to arterial blood gases, plasma glucose concentration, and blood pressure control during and after ischemia were met in all monkeys.

Table 1 summarizes neurological recovery in the groups. All monkeys subjected to 3 minutes of ischemia were neurologically normal at all times. Of the three animals subjected to 9 minutes of ischemia, two were completely normal at all times; the other monkey had mild upper extremity apraxia that persisted through only the second postischemic day, after which the neurological examination was completely normal. Animals subjected to 12 minutes of ischemia were obviously neurologically impaired after ischemia. The mean ± SEM neurological deficit score on the first day was 163 ± 54. These monkeys made steady neurological recovery such that gross neurological function was either normal (in two) or nearly normal (in two) in all by day 33 (Table 1). Normal neurological function was achieved earlier, by day 5, in one monkey. All persistent neurological deficits were referable to the upper extremities as apraxia, slight weakness, or decreased function. There were no persistent deficits in level of consciousness, cranial nerve function, or gait.

**Table 1. Neurological Deficit Scores for Monkeys After Ischemia**

<table>
<thead>
<tr>
<th>Postischemia day</th>
<th>Duration of ischemia (min)</th>
<th>12 (n=4)</th>
<th>9 (n=3)</th>
<th>3 (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>163±54</td>
<td>2±1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>91±28</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>55±26</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>43±28</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>36±16</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>23±11</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>28±13</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>15±6</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>11±4</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>8±3</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>9±3</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>6±4</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

**Figure 2.** Plot of nonmatching to sample test scores (% correct) for three groups of monkeys at various delay periods. There were no differences between groups subjected to 3 and 12 minutes of ischemia or between groups subjected to 3 and 9 minutes of ischemia. Time is not continuous on x axis.

Figure 2 details the nonmatching to sample test scores for the groups. All three groups scored above 70% at the 10-minute delay, with no differences between the 3- and 12-minute groups or between the 3- and 9-minute groups. The 3-minute (control) monkeys’ score on the 10-minute delay nonmatching to sample test (78%) was also similar to that of normal animals described in previous studies using cynomolgus monkeys (79%). The histopathological rank and the percentage correct on the 10-minute delay nonmatching to sample test for the animals with hippocampal damage (exposed to 12 minutes of ischemia) appeared to covary with the neurological deficit score (Table 2).

Monkeys subjected to 3 minutes of complete cerebral ischemia showed no evidence of neuronal loss in the hippocampus, temporal or parietal neocortex, basal ganglia, or thalamus. In one animal, a small focus of gliosis was seen unilaterally in the inferior putamen on GFAP stains. There was no gliosis in the hippocampus, neocortex, thalamus, or other portions of the basal ganglia. There was positive GFAP staining of the subependymal astrocytes and very faint, diffuse GFAP positivity in the subcortical and deep white matter in all three monkeys.

Animals subjected to 9 minutes of cerebral ischemia also had no apparent neuronal loss in the hippocampus, neocortex, thalamus, or basal ganglia. One monkey had mild to moderate gliosis on GFAP stains within the hilum of the dentate gyrus; the other two animals had mild to moderate gliosis on GFAP stains at 4 and 33 days.

**Table 2. Rank Order of Hippocampal Histopathology Score, Nonmatching to Sample Test Score, and Neurological Deficit Scores at 4 and 33 Days for Monkeys Exposed to 12 Minutes of Ischemia**

<table>
<thead>
<tr>
<th>Histopathology score rank</th>
<th>Nonmatching score (% correct)</th>
<th>Neurological deficit score 4 days</th>
<th>Neurological deficit score 33 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (least injury)</td>
<td>82</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>76</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>74</td>
<td>55</td>
<td>10</td>
</tr>
<tr>
<td>4 (most injury)</td>
<td>64</td>
<td>90</td>
<td>15</td>
</tr>
</tbody>
</table>
no gliosis in the hippocampus. Gliosis was not seen in the neocortex, thalamus, or basal ganglia. Subependymal astrocytes had positive GFAP staining in all three monkeys, and two had faint GFAP positivity in the white matter.

Results were more variable in the four animals with 12 minutes of cerebral ischemia. Neuronal loss was apparent by Nissl stain in the hippocampus of two monkeys. Both showed a focal loss of hippocampal pyramidal cells at the CA1/CA2 junction. In one animal the neuronal loss was barely perceptible, while in the other there was a focal, but distinct, area of cell loss. In the other two monkeys the hippocampal neuronal population appeared intact by Nissl stain. Neuronal loss was not apparent in other cortical regions, the thalamus, or the basal ganglia in any of the four animals. GFAP immunohistochemistry revealed gliosis in the hippocampus of all four. Reactive astrocytes were seen at the CA1/CA2 junction in the two monkeys with obvious neuronal loss, and a third showed focal gliosis in the CA2 region. Minimally detectable to mild gliosis was seen within the hilum of the dentate gyrus in three animals. There was positive staining of subependymal astrocytes and faint staining of the white matter in all four monkeys.

Sections of cervical spinal cord showed no evidence of injury. The gray matter was intact, with a normal population of motor neurons, and the white matter showed no evidence of demyelination or gliosis. The nerve roots were intact and of normal cellularity. No inflammation was seen in the spinal cord, nerve roots, or meninges. The leptomeninges had no fibrosis or chronic inflammation. Dura was not present in the sections but was grossly normal.

**Discussion**

Neurological dysfunction following global brain ischemia is a major source of morbidity after resuscitation. Impairment may take many forms, but one clearly recognized clinical entity is that of selective damage to the CA1 hippocampal neurons leading to short-term memory loss and learning impairment. Although a significant problem in humans, this type of neurological dysfunction has been difficult to model in animals. Most of the animal work attempting to uncover the function of the hippocampus and surrounding structures has been done using surgical ablation. Unfortunately, this method is too gross to destroy just CA1 neurons without harming adjacent brain. Also, neurological evaluation focusing specifically on CA1 function is difficult in animals. Recently, memory tests consisting of mazes and other apparatuses have been applied to rodents after ischemic neurological insults that selectively damage CA1 neurons, but learning or memory deficits in rodents are difficult to relate to the corresponding human clinical entities.

In an attempt to overcome some of these limitations, we subjected cynomolgus monkeys to various durations of global cerebral ischemia followed by prolonged neurological evaluation, neurobehavioral testing, and brain histopathologic analysis. We knew from previous work that 15 minutes of global cerebral ischemia in cynomolgus monkeys produced moderate to severe CA1 damage in all animals and significant quantifiable neurobehavioral deficits compared with historical control animals as well as severe gross neurological deficits in some animals. Thus, our primary intent was to define a dose-response relation between duration of ischemia and histopathological damage to the CA1 sector in this primate model. Secondly, we wanted to define the practical problems associated with neurological recovery and neurobehavioral testing in the postischemic period and, if possible, determine a duration of ischemia that would allow the monkey to cooperate fully with testing but that might also be associated with measurable cognitive deficits.

The primate model used in the present study was introduced in 1977. The model was soon found to be unreliable, due perhaps to failure to document a lack of brain blood flow during the ischemic period and to nonstandardized postischemic supportive care, aspects of peri-ischemic handling that were not appreciated at the time. This primate model of global cerebral ischemia has undergone modifications and is now thought to be highly reproducible and apparently predictive of clinical trials of neurotherapeutic agents in humans who have experienced cardiac arrest. For example, studies using this model have produced results that agree with the outcome of clinical trials of the calcium channel blockers nimodipine and lidoflazine. However, monkeys are typically evaluated neurologically for only 96 hours postischemia, and the neurological examination is heavily weighted toward motor, not cognitive, functions.

As stated, our primary goal was to determine the duration of global cerebral ischemia required to produce little or no gross motor dysfunction but with CA1 injury that might be expected to produce abnormalities on neurobehavioral testing. The data show that the critical duration is greater than 12 minutes in cynomolgus monkeys. Ischemic durations less than this (e.g., 9 minutes) were associated with no CA1 histopathologic changes. With an ischemic duration of 12 minutes, gross motor function, although markedly abnormal in the immediate postischemic period, became nearly normal in all animals. However, CA1 histopathological damage was mild to moderate, and this was not associated with demonstrable neurobehavioral decrements as measured by the delayed nonmatching to sample test compared with control animals. However, it is possible that the few monkeys tested precluded our finding a positive result because individual variation in the nonmatching to sample test was great. Nonetheless, the previously studied small group of animals (n=4) subjected to 15 minutes of global cerebral ischemia showed significant decrements on the same task compared with historical controls. Furthermore, monkeys exposed to 12 minutes of ischemia scored on average 75% correct on the nonmatching to sample test with a 10-minute delay, which is also comparable to the results in previously tested normal animals.

It is noteworthy that nearly all those monkeys subjected to 12 minutes of ischemia that demonstrated severe neurological impairment for a variable time in the postischemic period recovered normal neurological function (Table 2). In some animals this recovery took weeks. This agrees with previous observations by Miller and Myers, who noted that monkeys continued to improve neurologically for 30 days after global cerebral ischemia. This brings into question the wisdom of using only 96 hours of postischemic observation to evaluate...
neurotherapeutic modalities for use following global cerebral ischemia.11,13-15 However, neurological deficit scores at 96 hours, although not a specific measure of eventual neurological outcome, were a sensitive measure in that monkeys with the worst eventual outcome had the worst 96-hour neurological deficit scores. The reverse was also true; a good score at 96 hours was certainly predictive of good outcome, although, as noted, it would have been difficult to distinguish between monkeys on the basis of the gross neurological examination after 33 days. Thus, the 96-hour neurological deficit score appears to be a sensitive measure of eventual gross neurological function using this model. This has been a contention of those using this model and was formally postulated by Gisvold et al11 and would certainly appear to be supported by our data. However, it should be noted that, from the previous study of 15 minutes of ischemia, the pattern of neurological injury and recovery was not noticeably different from that seen with 12 minutes of ischemia. The mean±SD 4- and 33-day neurological deficit scores for the monkeys exposed to 15 minutes of ischemia were 55±44 and 6±12, respectively, compared with 50±33 and 6±8, respectively, for our 12-minute animals. Two of the 15-minute animals had normal neurological examinations at 33 days. Of the other two 15-minute animals, one was almost completely normal (neurological deficit score, 5 at 33 days) and the other had some very mild upper extremity apraxia (neurological deficit score, 25 at 33 days).6 As a group, these monkeys scored significantly worse on the nonmatching to sample test with a 10-minute delay than normal historical controls, suggesting that cognitive function cannot be predicted accurately by the gross neurologic examination using this model, regardless of the length of the postischemic observation period.8 On the other hand, cognitive function as measured by the percentage correct on the nonmatching to sample test with a 10-minute delay and the histopathologic rank appeared to covary with the neurological deficit score at 4 and 33 days in our monkeys exposed to 12 minutes of ischemia. That is, our 12-minute animal with the most histopathologic damage also had the lowest percentage correct and the highest neurological deficit score at 4 and 33 days. Conversely, our 12-minute animal with the least histopathologic damage also had the highest percentage correct and the lowest neurological deficit score at 4 and 33 days. The few monkeys studied (n=4) precludes any more detailed statistical correlation (Table 2).

Of concern was the pattern of neurological injury observed. Neurological deficits were all referable to the upper extremities. There appeared to be no cranial nerve deficits, lower extremity abnormalities, or level of consciousness abnormality in any monkey after the first few days postischemia. We were suspicious that cervical spinal cord ischemia or brachial plexus neuropathy may have occurred during tourniquet inflation. However, examination of two cervical spinal cords did not indicate any obvious pathology. It is possible that tourniquet inflation and hypotension selectively injured nerve roots or peripheral nerves that were not examined.

On the basis of these data, we conclude that cognitive neurological dysfunction can be studied in primates after global brain ischemia with nonmatching to sample neurobehavioral testing. The minimum duration of ischemia necessary to produce moderate CA1 histopathologic changes and demonstrable neurobehavioral deficits in a small group of animals is greater than 12 minutes in cynomolgus monkeys and, based on our previous work, is probably close to 15 minutes. This duration of ischemia, although survivable, is associated with gross neurological deficits that may persist for weeks after ischemia, requiring intensive care until their eventual resolution.

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